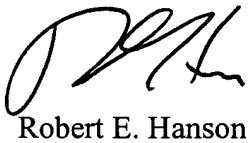
D. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. The examiner is invited to contact the undersigned (512)536-3085 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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Reg. No. 42,628
Attorney for Applicants

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600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201

Date: May 4, 2004

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Gang Chen, et al.

Serial No.: 09/699,023

Filed: October 27, 2000

For: ISOLATION OF BINDING PROTEINS WITH HIGH AFFINITY TO LIGANDS

Group Art Unit: 1632

Examiner: Ford, V.

Atty. Dkt. No.: UTSB:675US

CERTIFICATE OF MAILING
37 C.F.R. §1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231, on the date below:

05/04/04
Date

Robert E. Hanson

DECLARATION OF JONGSIK GAM UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, JONGSIK GAM, HEREBY DECLARE AS FOLLOWS:

1. I am a citizen of South Korea, and currently reside at 1014 Stratford Ct., State College, PA.

2. I was employed by The University of Texas from 1997 until 2004, with the title of Research Assistant. During that time I was a graduate student at The University of Texas. My primary duties as a Research Assistant were in conducting scientific research in the area of

pharmacy. One of my two supervisors was Brent Iverson, Ph.D., who is a co-inventor of the above captioned patent application.

2. I will receive a Ph.D. in Pharmacy from The University of Texas in May, 2004. I have been conducting research in the area of pharmacy since 1997. I have a Bachelors degree and a Masters degree in Pharmacy from the Seoul National University in Seoul, South Korea.

3. I have reviewed the amended claims of the above-captioned patent application and am familiar with the technology and steps described in the claims.

4. I understand that the Patent and Trademark Office Examiner in charge of assessing the patentability of the referenced patent application has rejected the claims of the above patent application for failing to recite a wash step. In particular, it is my understanding that the Examiner asserts that a separate wash step must be added to make the following procedure functional:

> 1. (Current amended) A method of obtaining a bacterium comprising a nucleic acid sequence encoding a binding protein capable of binding a target ligand comprising the steps of:
> (a) providing a Gram negative bacterium comprising a nucleic acid sequence encoding a candidate binding protein, wherein said binding protein is expressed in soluble form in the periplasm of said bacterium;
> (b) contacting said bacterium with a labeled ligand capable of entering said periplasm; and
> (c) selecting said bacterium based on the presence of said labeled ligand within the periplasm, wherein said ligand and said candidate binding protein are bound in said bacterium.

5. I am providing the present Declaration to submit data demonstrating that a wash step is not required for the successful use of the above procedure.

6. The studies carried out by me demonstrating that a wash step is not necessary for the function of the technique described in the claims can be summarized as follows:

A. Bacteria

E. coli ABLE™C and TG1 bacteria were obtained expressing a digoxigenin-specific scFv in soluble form in the periplasm. Periplasmic expression was achieved by linking the scFv to a *pelB* leader sequence. Expression was regulated by the *lac* inducible promoter, the expression of which is induced by the addition of IPTG to growth media.

B. Cell culture

Growth media was supplemented with ampicillin (100 μg/ml) when necessary. Bacteria containing the plasmid encoding the scFv were grown overnight in TB broth containing 2% glucose at 30°C. Cells were subcultured in fresh 2xYT (or LB) media and re-grown at 37°C for 2 hours until an OD600 of 0.5-0.8 was reached. The cells were induced with IPTG (0.2 mM) for an additional 4 hours at 25°C to express the scFv antibodies in the periplasm of the *E. coli* strains.

C. Labeling

50 μl of the induced cell cultures were incubated in 950 μl of 1xPBS at room temperature for 1 hour with BODIPY-conjugated digoxigenin (usually 200 nM = 0.2 μM). The incubation was performed successfully with and without use of permeabilizers (PMBN, EDTA or lactic acids were used when a permeabilizer was included). At this stage, trials were run both with and without a washing step after the incubation. The resulting cell suspension (10 μl without a washing step), was diluted with 1 ml of 1xPBS in a SIP tube for the FACS analysis.

D. Selection

10^4 events were analyzed on a Becton Dickinson FACSCalibur for both the washed and unwashed trials. Sheath flow was 1xPBS for all of the screens. Cell viability was judged using the propidium

iodide (PI) staining method (1 μg/ml). The screening demonstrated a high FACS signal relative to negative controls lacking the digoxigenin-specific scFv both with and without the wash step. The results confirmed that in cells expressing the scFv binding protein with specific affinity for the labeled ligand the bound complex was accumulated and becomes detectable above background levels (the control) based on this concentration of signal regardless of the presence of unbound labeled ligand.

7. Based on the foregoing studies, a wash step is not required for the use of the method described in the claims of this patent application to obtain a bacterium comprising a nucleic acid sequence encoding a binding protein capable of binding a target ligand.

8. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/4/04

Date

Jongsik Gam

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diffuse[1,adjective]
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Main Entry: **[2]dif·fuse**
Pronunciation: di-'fyüz
Function: *verb*
Inflected Form(s): **dif·fused**; **dif·fus·ing**
Etymology: Middle English *diffused,* pp., from Latin *diffusus,* past participle
transitive senses
1 a : to pour out and permit or cause to spread freely **b :** EXTEND, SCATTER **c :** to spread thinly or wastefully
2 : to subject to diffusion; *especially* **:** to break up and distribute (incident light) by reflection
intransitive senses
1 : to spread out or become transmitted especially by contact
2 : to undergo diffusion
- dif·fus·ible /di-'fyü-z&-b&l/ *adjective*

For **More Information on "diffuse" go to Britannica.com**

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Pronunciation Symbols

Definition

diffuse [Show phonetics]
verb [I or T]
1 to (cause something to) spread in many directions:
Television is a powerful means of diffusing knowledge.

2 to (cause a gas or liquid to) spread through or into a surrounding substance by mixing with it:
*Oxygen diffuses from the lungs **into** the bloodstream.*
The drop of red dye diffused slowly in the water.

diffuse [Show phonetics]
adjective
1 spread out and not directed in one place:
a diffuse light
The company has become large and diffuse.

2 DISAPPROVING not clear or easy to understand:
a diffuse literary style

diffusely [Show phonetics]
adverb

diffuser, diffusor [Show phonetics]
noun [C]
a device which is used to make light less direct, especially one used with a fluorescent light

diffusion [Show phonetics]
noun [U]
the process of diffusion in gases/liquids/solids

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